

<http://dx.doi.org/10.4314/ajtcam.v12i1.14>EVALUATION OF ACUTE TOXICITY AND ANTI-INFLAMMATORY EFFECTS OF *BACCHAROIDES SCHIMPERI* (DC.) IN EXPERIMENTAL ANIMALS

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Abstract

Background: Steroidal and non-steroidal anti-inflammatory drugs are most commonly used to treat inflammation, and shown to have severe side effects. In this study, we aimed at evaluating the anti-inflammatory and acute toxicity effects of *Baccharoides schimperi* (DC.) in order to get new anti-inflammatory agents of natural origin.

Materials and methods: The aerial part of the plant was dried under shade, ground and extracted with 96% alcohol (BSE). It was further fractionated in sequence to *n*-hexane (BSH), chloroform (BSC) and methanol (BSM) soluble fractions.

Acute toxicity was evaluated by oral administration of plant and hind paw induced-edema method in rats was used for the anti-inflammatory evaluation.

Results: The BSE was found safe up to the dose level of 3 g/kg b.w. and showed LD₅₀ value 7.250 g/kg body weight (b.w.) in mice. BSE showed significant anti-inflammatory effect (62.91%) at 500 mg/kg b.w. Further the *n*-hexane, chloroform and methanol fractions of BSE were tested for anti-inflammatory activity. The *n*-hexane fraction (BSH) exhibits significant activity (64.87%) at 400 mg/kg b.w. The methanol fraction (BSM) showed dose dependent activity, highest activity (60.42%) was observed at higher dose 400 of mg/kg b.w. In chloroform fraction (BSC) no significant activity was observed.

Conclusion: The results of the study revealed that the plant is safe to the experimental model and recommended as a potential source of anti-inflammatory agent.

Key words: Acute toxicity, anti-inflammatory activity, *Baccharoide schimperi* (DC.)

Introduction

Inflammation is a response of living cells and tissues to injury and usually comprises a wide array of enzyme activation, mediator release, extravasations of fluid, cell migration, tissue breakdown and repair (Perianayagam et al., 2006). Pain is frequently associated with inflammation (White et al., 2005; Cunha et al., 2008). Steroidal and non-steroidal anti-inflammatory drugs are the most commonly used to treat inflammation (Hassan et al., 2012), but both are prone to presenting with serious adverse reactions (Dharmasiri et al., 2003; Park et al., 2004).

Therefore, the search for new and cost effective anti-inflammatory and analgesic agents with little or no side effects for mankind is necessary. Over the years, medicinal plants have been an important source of new chemical substances with potential therapeutic effects. Hence, the investigation of new plants currently used by folk healers as pain relievers and anti-inflammatory agents should therefore be viewed as a fruitful and logical research strategy (Elisabetsky et al., 1995; Gupta et al., 2006). *Baccharoides schimperi* DC. (Syn. *Vernonia schimperi* DC.) belong to the family Astraceae and is largely distributed in the southern part of Saudi Arabia. Literature reports on *Baccharoides schimperi* DC. do not show any pharmacological work. Previous phytochemical screening on *Vernonia schimperi* (DC.) (Syn. *Baccharoides schimperi* (DC.)) exposed the presence of tannins, cardiac glycoside, sterols, alkaloids, phenols, saponins and glycosides (Ahmed et al., 2014).

In literature, other species of the genus *Vernonia kotschyana* Sch. Bip. ex Walp. (*Baccharoides adoensis* var. *kotschyana* (Sch. Bip. ex Walp.) roots were reported to have ethno-medicinal uses in variety of ailment including gastrointestinal disorders and wound healing effects. The active principles were found as acidic polysaccharide fractions, having pectic arabinogalactan type II structures (Nergard et al., 2004). Another species *Baccharoides lilacina* have been reported to have essential oils and the main constituents were identified as β -caryophyllene (27.7%), epi- α -cadinol (25.1%), caryophyllene oxide (9.9%), α -muurolol (7.6%), α -cadinene (6.1%), and α -cadinol (4.5%). The oil was found to be rich in oxygenated sesquiterpenes (47.1%) and sesquiterpene hydrocarbons (46.2%) (Joshi, 2013).

In view of the above medical properties associated with other species of the genus, *Baccharoides schimperi* DC. was investigated for *in-vivo* anti-inflammatory and acute toxicity work. In the present paper we are reporting the anti-inflammatory activity, LD₅₀ value and acute toxicity of the title plant.

Material and methods

Plant material

The plant material was collected in February, 2007 from Bani Malik, Saudi Arabia. The collected plant material was identified by the taxonomist Dr. M. Yusuf and a voucher specimen of the plant (# 15044) was deposited at the herbarium of the College of Pharmacy, King Saud University, Riyadh, Saudi Arabia.

Extraction process

The aerial part of the plant was dried under shade and ground. The material (200 g) was extracted in 96% ethanol (1 L \times 3) at room temperature for 72 h (24 h \times 3). The extract was filtered through filter paper (Whatman No.1) and solvent was evaporated to dryness at 40 °C under reduced pressure using Buchi Rotavapour and yielded green solid mass 9.4 g (4.7%) and labeled as BSE. The ethanol extract (BSE) 4.9 g was further fractionated in sequence at room temperature for 72 hr (24 hr \times 3) with *n*-hexane, chloroform and methanol (100 mL \times 3) and the solvent in each case

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was evaporated as described above, afforded *n*-hexane fraction (BSH) 0.280 g (5.7%), chloroform fraction (BSC) 1.6 g (32.7%) and methanol fraction (BSM) 2.9 g (59.1%). All samples were kept in air tight bottle at 4 °C in refrigerator for further use.

Animals

Wistar albino rats roughly the same age, weighing 180-200 g b.w. and Swiss albino mice (25-30 g b.w.) of either sex were procured from the Experimental Animal Care Center, College of Pharmacy, King Saud University, Riyadh and used in these studies. The animals were kept at constant temperature ($22 \pm 2^\circ\text{C}$), humidity (55%) and light-dark conditions (12/12 hr light/dark). They were provided with Purina chow and free access to drinking water *ad libitum*.

Acute toxicity study and LD₅₀

The acute toxicity test was performed on mice using oral route. BSE was dissolved in distilled water and administered at various doses, ranging from (500–10000 mg/kg b.w), to different groups of mice (each group contains six animals). The animals were observed for 24 hr for any behavioral change, symptoms of toxicity and mortality. Finally LD₅₀ was calculated by Karbar's method.

Carrageenan-induced rat paw edema test

Experimental inflammation was induced according to the method described by Winter et al., 1962. Briefly, 0.05 mL of 1% carrageenan sodium salt was injected into the right hind foot of each rat under the plantar aponeurosis. The test groups (six animals) of rats were treated orally with ethanol extract (BSE) 250 and 500 mg/kg b.w, BSH 400 mg/kg b.w, BSC and BSM, 200 and 400 mg/kg b.w, 1 hr before the carrageenan injection. At the same time, the control group (six animals) was given normal saline 5 mL/kg and the reference group (six animals) was administered an aqueous solution of Phenylbutazone 100 mg/kg b.w orally. The measurements of paw volumes were done by the displacement technique using a plethysmometer (Apelex, France) immediately and 3 hr after the injection of carrageenan. The inhibitory activity was calculated according to the following formula:

Percent inhibition = $100 [1 - (a-b/c-d)]$

where 'a' and 'b' is the mean paw volume of treated rats after and before carrageenan injection respectively and whereas, 'c' and 'd' are the mean paw volume of control rats after and before carrageenan injection respectively.

Statistical analysis

Values are given as arithmetic means \pm standard error of the mean (S.E.M.). Data was statistically analyzed by using one-way analysis of variance (ANOVA) followed by Student's t-test.

Results

Acute toxicity test

In the acute toxicity assay no deaths were observed during the tested dose of up to 3000 mg/kg b.w of BSE, whereas at the doses of 4000, 8000 and 10000 mg/kg b.w., mean mortality was observed as 0.5, 1.5 and 3 respectively (Table I). The median lethal dose (LD₅₀) of BSE was calculated by Karbar's method and found to be 7.250 g/kg. During this experiment, behavioral changes like writhing, micturition, defecation, sedation and increase respiration rate were observed at higher doses 4000, 8000 and 10000 mg/kg b.w. but at 2000 mg/kg b.w. beside sedation, defecation and writhing were also observed.

Table 1: LD₅₀ determination of BSE by Karbar's method

Group	Dose mg/kg	Dose Difference (a)	Dead	Mean Mortality (b)	Product (a*b)
1	500	-	0	-	-
2	1000	500	0	-	500
3	2000	1000	0	-	1000
4	4000	2000	1	0.5	1000
5	8000	4000	2	1.5	6000
6	10000	2000	4	3	8000
					16500

$$\text{LD}_{50} = 1000 - (16500/6) = 7250 \text{ mg}$$

Carrageenan-induced rat paw edema

The anti-inflammatory activity of BSE was measured at the dose of 250 and 500 mg/kg b.w. and its fractions BSH was measured at 400 mg/kg b.w. BSC and BSM were measured at the dose of 200 and 400 mg/kg and the results are summarized in Table 2. The BSE showed significant anti-inflammatory activity, 62.91% inhibition at 500 mg/kg b.w. and the results are comparable to that of phenylbutazone standard anti-inflammatory drug. While among the fractions i.e. BSH, BSC and BSM the BSH showed good anti-inflammatory activity, 64.87% inhibition at the dose of 400 mg/kg b.w. Conversely, the BSC did not show any significant activity at both higher and lower doses. The BSM showed negligible activity at lower dose but showed significant activity, 60.24% inhibition at higher dose (400 mg/kg b.w.) and are comparable to that of standard drug phenylbutazone. Abrupt rise in the activity at higher dose of BSM prompted us to determine the activity at 300 mg/kg b.w. and found 41.71 % inhibition which suggested that VSM showing dose-dependent activity.

Table 2: Anti-inflammatory effects of *Baccharoide schimperi* (DC.) against carrageenan induced paw edema in rats

Treatment	Dose mg/kg	Before Carrageenan	1 Hour	2 Hours	3 Hours	Increase after 3 hours	% Inhibition
Carrageenan	0.05 ml of 1%	1.04±0.04	1.91±0.07	2.27±0.07	2.13±0.10	1.09±0.11	
PBZ+ Carrageenan	100	1.09±0.04	1.25±0.02	1.32±0.02	1.29±0.03	0.20±0.03***	81.61
BSE+ Carrageenan	250	1.07±0.03	1.47±0.26	1.67±0.08	1.69±0.03	0.62±0.05*	36.77
BSE+ Carrageenan	500	1.02±0.05	1.49±0.03	1.61±0.04	1.45±0.03	0.40±0.02***	62.91
BSH+ Carrageenan	400	0.97±0.03	1.42±0.03	1.44±0.01	1.35±0.01	0.38±0.02***	64.87
BSC+ Carrageenan	200	1.03±0.03	1.68±0.02	1.85±0.01	1.94±0.03	0.91±0.04	15.64
BSC+ Carrageenan	400	1.01±0.03	1.76±0.03	1.83±0.02	1.88±0.03	0.89±0.03*	17.79
BSM+ Carrageenan	200	1.02±0.03	1.62±0.02	1.83±0.02	1.93±0.04	0.90±0.06	16.87
BSM+ Carrageenan	300	1.01±0.04	1.63±0.01	1.64±0.02	1.64±0.06	0.63±0.05	41.71
BSM+ Carrageenan	400	1.00±0.03	1.53±0.02	1.50±0.02	1.43±0.01	0.43±0.04***	60.42

*P<0.05, *** P<0.001 one-way analysis of variance (ANOVA) followed by Student's t-test, PBZ = Phenylbutazone, BSE = Ethanol extract, BSH = Hexane fraction, BSC = Chloroform fraction, BSM = Methanol fraction

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Discussion

The present study showed the anti-inflammatory activity and toxicity results of *Baccharoides schimperi* (DC.). BSE showed encouraging results that's why it was further fractionated in sequence to *n*-hexane, chloroform and methanol soluble fractions and tested for anti-inflammatory activity to get the active fraction. Toxicity is the degree to which a substance is poisonous (Martin, 2002); evaluation of crude plant drugs must be included its possible toxicity and/or safety margin (Ibrahim et al., 2007). In this study the ethanol extract (BSE) was not toxic to animals up to a dose of 3g /kg b.w., presenting LD₅₀ value 7.250 g/kg b.w. Carrageenan-induced edema is a common and suitable procedure to screen the anti-inflammatory activity in experimental animal model for acute inflammation and is believed to be biphasic (Vinegar et al., 1969). The early phase (1–2 hr) of the carrageenan model is mainly mediated by histamine, serotonin and increased synthesis of prostaglandins in the damaged tissue surroundings. The late phase is sustained by prostaglandin release and mediated by Bradykinin, leukotrienes, polymorphonuclear cells and prostaglandins produced by tissue macrophages (Brito and Antonio, 1998; Gupta et al., 2006).

The inhibitory activity showed by the ethanol extract (BSE) of *B. schimperi* (500 mg/kg b.w) over a period of 4 hr in carrageenan-induced paw inflammation was quite similar to that of exhibited by the group treated with phenylbutazone. Among the BSE fractions the *n*-hexane fraction, BSH, (400 mg/kg b.w) showed more inhibition in comparison of BSE itself. The methanol fraction, BSM, (200, 300 and 400 mg/kg b.w) showed the dose-dependent activity and increases as the dose increases and highest activity was recorded at the highest dose used as shown in Table 2. The chloroform fraction does not show any significant activity. The results shown in Table 2 clearly indicated that the anti-inflammatory active compounds in the plant extract distributed among the *n*-hexane fraction (BSH) and methanol fraction (BSM).

Furthermore, variety of *in-vitro* and *in-vivo* experiments have shown that flavonoids, tannins, triterpenoids and other secondary plant metabolites possess analgesic and anti-inflammatory properties (Yuan et al., 2006; Salminen et al., 2008; García-Lafuente et al., 2009). Flavonoids inhibit phosphodiesterases, which are involved in cell activation in the biosynthesis of protein kinases (regulatory enzymes in inflammation) (Manthey et al., 2001). The flavonoid luteolin from *vernonia patula* (Lin and Wang, 2002) have been reported to inhibit the activity of cyclooxygenase and lipoxygenase, decrease the level of PGE₂ and the release and expression of cyclooxygenase-2 (COX -2) (Shimoi et al., 2000). Tannins are reported as potent inhibitors of cyclooxygenase-1 and also possessed anti-phlogistic activity (Pan et al., 2010). Again the phenolic compounds have also been found to exhibit substantial anti-inflammatory effects against carrageenan-induced paw inflammation by inhibition of leukocyte migration, reduction of serum lysozyme level, NO and PGF₂ (Wu et al., 2006).

The previous phytochemical work done on *V. schimperi* (Ahmed et al., 2014) revealed that the *n*-hexane fraction contains glycosides, sterols and phenols on the other hand high polar organic solvent fractions like acetonitrile and methanol fractions contains saponins, tannins, flavonoids, glycosides, cardiac glycosides and phenols. The anti-inflammatory activity of BSH and BSM may be attributed due to the presence of these phytochemicals in *n*-hexane and methanol fractions. Insignificant inhibitory activity of BSC may be due to presence of some compound having antagonistic effect.

Conclusion

The findings of the present work showed that the plant is safe to the experimental model and recommended as a potential source of anti-inflammatory agent. Moreover, further work is required to find out the active anti-inflammatory constituents of the plant.

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